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# MODELS OF ALLOSTERIC ENZYMES AND THEIR ANALYSIS BY THE GRAPH THEORY METHOD

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# MODELS OF ALLOSTERIC ENZYMES AND THEIR ANALYSIS BY THE GRAPH THEORY METHOD

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Using the very simple example of a dimer protein, possible equilibrium cooperative models intended for description of the properties of allosteric enzymes were considered. Together with indirect cooperation, involved in the model of Monot et al. [1], the "direct" cooperation arising as a result of direct interaction of active centers was investigated. Computations were made for the influence of an allosteric inhibitor and a competitive activator on the activity of an enzyme. The results of the computations made by the graph theory method proposed in an earlier study are in qualitative agreement with the data of Gerhart and Pardee for aspartate transcarbamylase. The proposed models are described by equations containing fewer constants than the model of Monod et al.

The kinetics of allosteric inhibition and activation of a number of enzymes now have been investigated. /679* The principal characteristics of allosteric enzymes have been determined as a result of these studies: 1) allosteric protein contains two or a greater number of active centers; 2) these centers are equivalent -- the protein therefore has a symmetrical quaternary structure and is a symmetrical oligomer; 3) the kinetics of interaction of a protein with a substrate and with allosteric effectors is cooperative, which is manifested in the presence of a "knee" in the curve of the dependence of the rate of reaction on the concentration of the substrate, and accordingly in the curve of saturation of the enzyme by the substrate. This cooperation may be destroyed as a result of various influences on a protein molecule. The presence of cooperation means a dependence of the interaction in a particular active center on the status of the other active centers, on whether they are occupied (and specifically by what) or free.

Hemoglobin is a well-studied example of an allosteric protein. In a recent study by Monod et al. [1] they propose a model of an allosteric protein and investigate its properties. The model is a symmetrical oligomer consisting of n protomers. Therefore, there are n equivalent active centers. It is assumed that the oligomer may be in two or more different states, differing in their affinity to the substrate and effectors. The model therefore is cooperative: because the joining of one molecule of the substrate is characterized by a different affinity and different state of the protein, the equilibrium between these states is displaced as a result of such joining and a second molecule of the substrate interacts with the protein, already changing. Therefore, the joining of the substrate to a particular active center exerts an influence

*/Numbers in the margin indicate pagination of the original foreign text.

on joining in other active centers. Monod and his associates [1] demonstrate that the model leads to results qualitatively in agreement with the results of investigation of a number of allosteric enzymes and hemoglobin.

The simplest model, possessing the above-mentioned properties, is a dimer, obviously possessing a second-order axis of symmetry. The region of interaction of the two protomers consists of two identical sets of functional groups (to use Monod's terminology [1], this is an isologic dimer). We will assume, going along with the mentioned authors, that the protein may be in two states: R and T. Each of them has three substates: $R_{00}$, $R_{10} = R_{01}$, $R_{11}$, and accordingly, $T_{00}$, $T_{10} = T_{01}$, $T_{11}$ (two active centers, 0 -- a free center and 1 -- a center occupied by a molecule of the substrate S). The equilibrium conditions are:

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$$T_{00}=LR_{00}$$
$$R_{10}=2R_{00}\frac{S}{K_R};\quad R_{11}=\tfrac{1}{2}R_{10}\frac{S}{K_R}=R_{00}\frac{S^2}{K_R^2}$$
$$T_{10}=2T_{00}\frac{S}{K_T};\quad T_{11}=\frac{1}{2}T_{10}\frac{S}{K_T}=T_{00}\frac{S^2}{K_T^2} \qquad (1)$$

The total quantity of enzyme is

$$E=R_{00}+R_{10}+R_{11}+T_{00}+T_{10}+T_{11}. \qquad (2)$$

Here $K_R$ and $K_T$ are the dissociation constants in the states R and T, and L is the equilibrium constant for the transition $R_{00} \leftrightarrow T_{00}$. It is assumed that the dissociation constant is the same in $R_{10}$ and $R_{11}$ (and, accordingly, in $T_{10}$ and $T_{11}$). The saturation function by the substrate is

$$\bar{Y}=\frac{R_{10}+2R_{11}+T_{10}+2T_{11}}{2(R_{00}+R_{10}+R_{11}+T_{00}+T_{10}+T_{11})}=$$
$$=\frac{K_R\frac{1+Lc}{1+Lc^2}S+S^2}{K_R^2\frac{1+L}{1+Lc^2}+2K_R\frac{1+Lc}{1+Lc^2}S+S^2}=\frac{1}{2}\frac{d\ln Q}{d\ln S}, \qquad (3)$$

where $c = K_R/K_T$. When $c = 1$, or when $L \to 0$, cooperation disappears; $\bar{Y} = \frac{S}{K_R+S} \cdot Q$ is the denominator of (3).

The corresponding expression for the rate of the reaction, obtained from the equilibrium conditions, has the form

$$v = 2Ek_4 \frac{1+\varkappa Lc^2}{1+Lc^2} \cdot \frac{K_R \frac{1+\varkappa Lc}{1+\varkappa Lc^2} S + S^2}{K_R^2 \frac{1+L}{1+Lc^2} + 2K_R \frac{1+Lc}{1+Lc^2} S + S^2}, \quad (4)$$

where $k_4$ is the rate constant for the states $R_{10} = R_{01}$ and $R_{11}$, and $\varkappa k_4$ for the states $T_{10} = T_{01}$ and $T_{11}$.

It is possible to consider a model alternative to the Monod model [1], not assuming the existence of several states R, T..., but only one state F with internal cooperation. Constants of the type $K_R$ and $k_4$ are assumed to be different for $F_{10} = F_{01}$ and $F_{11}$, in other words, the joining of one molecule S exerts a direct influence on the interaction of a second molecule S with protein. Such a model has a graphic physical sense -- the joining of S changes the quaternary and possibly the tertiary and secondary structure of the protein. Obviously, we refer here to cooperation of a different type, which we will call direct cooperation, in contrast to indirect cooperation, investigated by Monod et al. [1] (see [2]).

An earlier study [3] considered a corresponding example -- a symmetrical dimer with direct cooperation. In this case the saturation function has the form

$$\bar{Y} = \frac{K_2 S + S^2}{K_1 K_2 + 2K_2 S + S^2} = \frac{1}{2} \frac{d \ln Q}{d \ln S}, \quad (5)$$

and the rate

$$v = 2Ek_2 \varkappa \frac{\frac{K_2}{\varkappa} S + S^2}{K_1 K_2 + 2K_2 S + S^2}, \quad (6)$$

where $k_2$, $K_1$, $K_2$ have the same sense as in [3], $\varkappa = k_4/k_2$. Expressions (5) and (6) have a simpler form than (3) and (4) respectively and contain one less constant. At the same time, they lead to similar qualitative results. The quantitative results, to be sure, differ. For example, the value S in the case of semi-saturation, that is, when $\bar{Y} = 0.5$, in accordance with formula (3), is

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$$S_{0.5} = K_R \sqrt{\frac{1+L}{1+Lc^2}} \quad (7)$$

according to formula (5),

$$S_{0.5} = K_1 \sqrt{\eta}, \quad (8)$$

where $\eta = K_2/K_1$.

Computation of the equilibrium model obtained by Monod et al. can be done easily by the graph theory method [3]. This model assumes the presence in a molecule of the enzyme of states existing in equilibrium in pairs, and thus describes a special case of a stationary enzymatic reaction.

The model of stationary enzymatic reactions requires determination of the equilibrium of each state of the enzyme with several other states. Therefore, the theory of graphs, developed for stationary processes, also is applicable for equilibrium processes. As an example Figure 1 shows the graph for a dimer equivalent to the system of equations (1) and graphically representing the possible transitions. Here $b/k_3 = K_R$, $a/k_1 = K_T$, and $k_-/k = L$. The arrows annotating P show the release of a product with the corresponding rate constant $k_2$ or $k_4$. The rate of reaction (4) can be obtained from an analysis of the graph. Paired equilibrium leads to the appearance of cooperative transitions, that is, transitions dependent on one another. Such dependent transitions in Figure 1 are the transitions $R_{11} \leftrightarrow T_{11}$ and $R_{10} \leftrightarrow T_{10}$. They are represented by the dashed arrows and may be neglected when computing the graph.

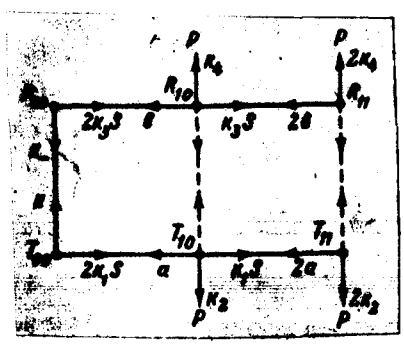


Figure 1. Graph for a Dimer.

The state T or the state R in Figure 1 each can be considered separately as a model of a dimer with direct cooperation [2] if it is taken into account that the second molecule of the substrate is sorbed in the enzyme differently from the first. Thus, the model of Monod et al., taking into account equilibrium and without the joining of a link contains a greater number of constants and states than a model without such additional equilibrium.

Now we will consider a model of an allosteric enzyme, assuming that the states of the enzyme change only as a result of the joining of a link. If only paired equilibriums are taken into account, we obtain simple cooperative models/682 with direct and indirect cooperation. Analysis will be made using the graph theory method.

Allosteric inhibition. Now we will consider a model of an isologic dimer with two equivalent active centers in relation to the substrate S and two centers in relation to the inhibitor I. We will assume that the enzyme can be in three equilibrium states F, F' and F", where F is an enzyme free of the inhibitor, F' is one center occupied by an inhibitor, and F" are both centers occupied by an inhibitor.

In each of these states a molecule of the enzyme can sorb one or two molecules of the substrate on active centers. In addition, we take into account the direct cooperation during the joining of the substrate in a state free of the inhibitor F. Together with the inhibitor in one (F') or in two (F") centers the enzyme loses direct cooperation for the joining of the substrate.

Figure 2 shows the graph for this model. Here F, $F_1$, and $F_2$ are the concentrations of the enzyme, free of the inhibitor, accordingly without a substrate, with one center, occupied by the substrate, and with two centers, occupied by the substrate. F', $F'_1$, $F'_2$ are the same for an enzyme with one

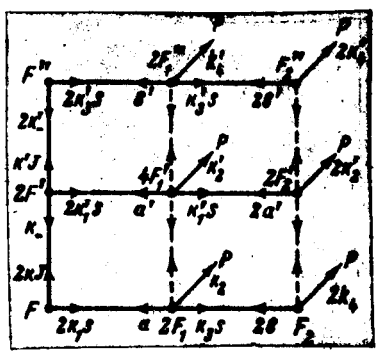

Figure 2. Graph for a Dimer with Inhibition.

center, occupied by an inhibitor, and F", $F''_1$, $F''_2$ -- with two centers, occupied by an inhibitor. The dashed line shows cooperative joinings of the inhibitor.

Now we will compute the rate $\nu$ of reaction, using the graph theory method described in the earlier paper [3]. We have

$$v = E\frac{\sum_i k_i D_i}{\sum_i D_i}. \qquad (9)$$

Here summation is carried out for all the nodes of the graph. We will simplify the graph using equilibrium relations. In order to compute each base determinant $D_i$ it now is necessary to consider only one "tree":

$$D = 2ba2a'a'2b'b'2k_-k_-$$
$$D' = 2ba2a'a'2b'b'2k_-2k_-l$$
$$D'' = 2ba2a'a'2b'b'2kk'\cdot l^2$$

$$D_1 = 2a'a'2b'b'2k'k'2b \cdot 2k_1 \cdot S$$
$$D_1' = 2ba2b'b'2k'2k2a'2k_1' \cdot IS'$$
$$D_1'' = 2ba2a'a'2kk'2k_2'2b'I^2S$$
$$D_2 = 2a'a'2b'b'2k'k'2k_1k_2S^2$$
$$D_2' = 2ba2b'b'2k'2k2k_1' \cdot k_1'IS^2$$
$$D_2'' = 2ba2a'a'2kk'2k_2'k_2'I^2S^2$$
$$v = 2ES\frac{\alpha}{\beta}, \qquad (10)$$

where

$$\alpha = a'^2b'^2k'k'k_1(k_2b + k_4k_2S) + 2abb'^2kk'k_1'k_2'(a' + k_1'S) \cdot I + aba'^2kk'k_2'k_4'(b' + k_2'S)I^2,$$
$$\beta = a'^2b'^2k'k'(ab + 2bk_1S + k_1k_2S^2) + 2abb'^2kk'(a' + k_1'S)^2 \cdot I + aba'^2kk'(b' + k_2'S)^2 \cdot I^2.$$

Now the derived expression will be analyzed. In the absence of an inhibitor (equilibrium is displaced to the state F), we obtain the cooperative kinetics (compare [2, 3]): /683

$$v_0 = 2ES\frac{k_2K' + k_4S}{KK' + 2K'S + S^2}, \qquad (11)$$

where $K' = b/k_3$, and $K = a/k_1$. When $K = K'$ and $k_2 = k_4$ (absence of cooperation); the usual Michaelis-Menten expression is

$$v_0 = 2E\frac{k_2S}{K+S}. \qquad (12)$$

In the case of a large excess of the inhibitor the equilibrium is displaced to the state F'' and cooperation disappears

$$v_{I\to\infty} = 2E\frac{k_4'S}{K'' + S}, \quad K'' = b'/k_3'. \qquad (13)$$

The maximum rate is

$$\nu_{max} = 2E\frac{a'^2b'^2k_-k'_-k_1k_4k_2 + 2abb'^2kk'_-k'_1k'_2k'_1I + aba'^2kk'k'_2k'_4k'_2I^2}{a'^2b'^2k_-k'_-k_1k_2 + 2abb'^2kk'_-k'^2_1 \cdot I + aba'^2kk'k'^2_2I^2} \tag{14}$$

If the catalytic activity of the enzyme in the states $F_2$, $F'_2$, and $F''_2$ is identical, $k_4 = k'_2 = k'_4$,

$$\nu_{max} = 2k_4E,$$

that is, is not dependent on the concentration of the inhibitor. Similar results were obtained by Gerhart and Pardee for aspartate transcarbamylase (ATCase), for which S is aspartic acid, and I is cytidine triphosphate [4]. If there is no direct cooperation, $k_1 = k_3$, $k_2 = k_4$, and a = b, with a constant concentration of the inhibitor we obtain the cooperative kinetics as a result of indirect cooperation

$$v = 2ES\frac{K_1 + K_2S}{K_3 + K_4S + K_5S^2}, \tag{15}$$

where the concentration of the inhibitor is included in the constants.

In the absence of direct and indirect cooperation, that is, when

$$\begin{aligned} k'_1 = k'_3 = k'_2 = k_3 \\ a = a' = b' = b \\ k_2 = k'_2 = k'_4 \end{aligned}$$

we obtain the Michaelis-Menten expression

$$v = 2E\frac{k_2S}{K+S}. \tag{16}$$

The degree of inhibition is

$$i = \frac{v_0 - v}{v} = \frac{\alpha - \beta}{\gamma + \beta}, \tag{17}$$

where

$$\alpha = ab[\ldots + k_2S)][2b'^2k_-(a' + k'_4S)^2\ldots]I,$$

[illegible]

If one type of cooperation remains, either indirect: /684

$$a' = b',\ a = b,\ k_1 = k_3,\ k_1' = k_3',\ k_2 = k_4,\ k_2' = k_4', \tag{18}$$

or direct:

$$a = a' = b' > b,\ k_1 = k_1' = k_3' < k_3,\quad k_2 = k_2' = k_4' = k_4, \tag{19}$$

we obtain an expression of the form

$$i = \frac{I(2k_-' + k'I)}{K_1 I(2k_-' + k'I) + K_2}, \tag{20}$$

where $K_1$ and $K_2$ include the concentration of the substrate. With condition (19), $K_1 = (ak_3S)^{-1}(ab + 2bk'S + k_1k_3S^2)$. In the case of large I

$$i_{I\to\infty} = \frac{1}{K_1} = \frac{ak_3S}{ab + 2bk_1S + k_1k_3S^2}. \tag{21}$$

Thus, the dependence i(I) is represented by a curve tending to saturation when $I \to \infty$. The value $i(I \to \infty)$ decreases with an increase of S.

$$i_{\substack{I\to\infty \\ S\to\infty}} = \frac{a}{k_1S} = \frac{K_T}{S}.$$

These results also are similar qualitatively to those obtained by Gerhart and Pardee [4]. However, the investigated model cannot be considered adequate for the system studied quantitatively by these authors. ATCase apparently contains four, rather than two active centers, which will be taken into account in subsequent computations. With respect to allowance for the second substrate in this system, its concentration apparently is included in the corresponding constants.

In the considered case both types of cooperation lead to equivalent results.

Effect of an activator. Now we will consider the action of the effector A, being an analogue of the substrate. Joining in one of the two active centers, the effector exerts an influence on the interaction of the protomers, increasing the affinity of the unoccupied active center to the substrate. At the

same time, in occupying the active centers the effector decreases the number of centers free for the substrate.

Therefore, in the case of small concentrations the effector should act as an activator, and in the case of large concentrations as an inhibitor. We will call such an effector a competitive activator.

Figure 3 shows a graph of the corresponding model. The dashed line shows the cooperative joining of an activator to the state of an enzyme, one center of which is occupied by the substrate.

We will consider three states of an enzyme with different filling of the centers by an activator: F -- an enzyme free from an activator, $F_A$ -- an enzyme, one center of which is occupied by an activator, $F_{AA}$ -- an enzyme, both centers of which are occupied by a competitive activator and which therefore forms an inactive complex.

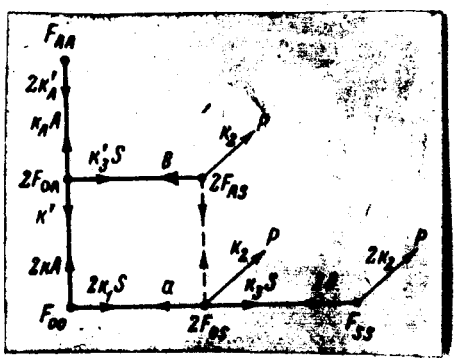


Figure 3. Graph for the Competitive Activation of a Dimer.

These three states are in paired equilibrium. The joining of the substrate to an enzyme existing in each of these states, if it is possible, displaces the equilibrium and indirect cooperation thereby is manifested. In the state F there can be joining of two molecules of the substrate. We will assume that it occurs with direct cooperation. The notations are as follows: $F_{00}$ is the concentration of the free enzyme; $F_{0S}$ is the concentration of an enzyme binding one molecule S; $F_{SS}$ is the concentration of an enzyme binding two molecules S in two active centers; $F_{0A}$ is the concentration of an enzyme binding one molecule A, $F_{AA}$ -- binding two molecules A; and $F_{AS}$ -- one center joins S and the other A. /685

It is not difficult to find the base determinants $D_{00}$, $D_{0S}$, $D_{0A}$, $D_{AA}$, and $D_{AS}$. We will assume as a simplification that cooperation is associated only with the difference of affinity of the enzyme to the substrate in different states with an identical catalytic activity, that is, the product is released from all the states with the identical rate constant $k_2$. Then the rate of reaction is:

$$v = 2k'_A k_2 ES \frac{k'k_1b'(b + k_3S) + abkk'_3 A}{b'k'k'_A(ab + 2bk_1S + k_1k_3S^2) + 2abkk'_A(b' + \ldots S)A + abb'k\ldots} \qquad (23)$$

We will consider the following four cases:

1) $k_3 = k'_3$ and $b = b'$ -- both cooperation mechanisms are present for the substrate;

2) $k_1 = k_3$ and $a = b$ -- only indirect cooperation is present for the substrate;

3) $k_1 = k_3 = k'_3$ and $a = b = b'$ -- cooperation for the substrate is absent;

4) $A = 0$ -- only direct cooperation is present.

In the first case the rate of the reaction is:

$$v = 2k'_A k_3 ES \frac{k'k_1(b + k_3S) + akk_3A}{k'k'_A(ab + 2bk_1S + k_1k_3S^2) + 2akk'_A(b + k_3S)A + abkk_AA^2}. \quad (24)$$

With large A, $v \to 0$, that is, A operates as an inhibitor. It is exactly in this way that maleate influences ATCase [4]. With an increase of A the curve $v(A)$ passes through a maximum, satisfying the condition

$$\frac{dv}{dA} = 0 \quad (25)$$

or

$$abkk_Ak_3A^2 + 2bk_Ak'k_1(b + k_3S)A + k'k'_Ak_3(k_1k_3S^2 + 2bk_1S - ab) \quad (25a)$$

When $k_3 \gg k_1$, $a \gg b$ the concentration A, corresponding to the maximum $v$,

$$A \cong K_A^{-1}\sqrt{1 - 2K_SS - K_{SS}S^2}, \quad (26)$$

where

$$K_A^{-1} = \sqrt{\frac{k'k'_A}{kk_A}}, \quad K_S = \frac{k_1}{a}, \quad K_{SS} = \frac{k_1}{a} \cdot \frac{k_3}{b}.$$

Obviously, the maximum can be observed only with small S. In the case of small concentrations of maleate, ATCase is activated [4]. In this case a maximum actually is observed on the curve of the dependence $v(A)$, disappearing after heating of the enzyme. If the heating destroys cooperation, that is, it is necessary to consider the third case, and if $k = k_A$ and $k' = k'_A$, (23) becomes

$$v = \frac{2k_3ES}{K + SKK'_AA}. \quad (27)$$

that is, A acts as an ordinary competitive inhibitor. This once again is in /686
qualitative agreement with the data for ATCase in [4]. When A = 0 we obtain direct cooperative kinetics (fourth case):

$$v = 2k_2E\frac{K'S + S^2}{KK' + 2K'S + S^2}. \quad (28)$$

If only indirect cooperation for the substrate (second case) is present we obtain from (23)

$$v = 2ES\frac{K_1 + K_2S}{K_3 + K_4S + K_5S^2}, \quad (29)$$

where the constants include the concentration of the activator.

Thus both types of cooperation also lead in this case to equivalent results.

## Conclusions

Using the very simple example of a dimer protein we considered possible equilibrium cooperative models intended for description of the properties of allosteric enzymes. Together with indirect cooperation, involved in the model of Monod et al. [1], we investigated the "direct" cooperation arising as a result of direct interaction of active centers. Computations were made for the influence of an allosteric inhibitor and a competitive activator on the activity of an enzyme. The results of the computations made by the graph theory method proposed in an earlier study are in qualitative agreement with the data of Gerhart and Pardee for aspartate transcarbamylase. The proposed models are described by equations containing fewer constants than the model of Monod et al.

## REFERENCES

1. Monod, J., J. Wyman and J. P. Changeux: J. Mol. Biol. 12: 88, 1965.
2. Vol'kenshteyn, M. V. et al.: IN: Molekulyarnaya Biofizika. (Molecular Biophysics.) "Nauka", 1965, p. 5.
3. Vol'kenshteyn, M. V. and B. N. Gol'dshteyn: Biokhimiya 31: 541, 1965.
4. Gerhart, J. C. and A. B. Pardee: Cold Spring Harbor Symposia 28: 491, 1963.

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